Remarks

Claims 45-86 are pending. Claim 64-81 are canceled. New claims 82 and 83 have been added. Applicants appreciate the Examiner's apparent withdrawal of the previous rejections imposed under 35 USC § 112.

Claim Rejections - 35 USC § 103

The examiner has imposed a rejection of all the claims under 35 USC 103(a) and has deemed all the claims to be obvious over Jablonka et al., Archivum Veterinarium Polnicum (1992) Vol. 32, pp 57-66, in view of Stoll et al. Annals N.Y. Accad. Sci. (1994), pp 122-128; Biewenga et al. Gen Pharm (1997) Vol. 29, pp 315-331; Sian et al. Annals of Neurology (1994) Vol 35, pp 348-355, and Kozhenivkova et al. Bull. Experimental Biol. and Med. (1999) Vol. 128, pp 535-537.

In response, the Examiner's attention is respectfully drawn to MPEP 2142, which indicates that to establish a *prima facie* case of obviousness, three basic criteria must be met.

1) There must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings.

2) Second, there must be a reasonable expectation of success.

3) The prior art references must teach or suggest all the claim limitations. (Notably, this section of the MPEP also requires that the reasonable expectation of success must be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991)).

Applicants respectfully submit that, for at least the following reasons, a *prima facie* case of obviousness has not been set forth in the present case.

At the outset, Applicants note that in imposing the present rejection, the Examiner relies in part on *In re Crockett* 126 USPQ 186 (CCPS, 1960), and adapts a quotation from this case to state:

> Assuming that [ambroxile, α-lipoci acid and ACE inhibitors] together produce an effect somewhat greater that the sum of their separate effects... claim to their joint use is not patentable.

In response, Applicants point out that the present invention does not demonstrate that the presently claimed compositions and methods produce an effect that is "*somewhat* greater than the sum of their separate effects." Rather, the record demonstrates that the presently claimed agents, when each is administered alone, have **no** effect on reduction of neuronal damage (see page 9, line 21, through line 4, page 10 of the present specification), while the combined administration of the claimed agents has a *significant and synergistic* effect (i.e., see the data in appended to the Declaration of Dr. Frank Striggow and submitted with the Applicants' previous response of December 12, 2006, as well as the present specification in Example 3). Therefore, the present invention is distinct from that which was deemed insufficient to overcome an obviousness rejection in *In re Crockett*.

With respect to the Examiner's assertion that the previous response of December 12, 2006, and the declaration of Dr. Frank Striggow are unpersuasive, Applicants note the Office Action response states the following:

> It is not persuasive because synergistic effect is enough for unobviousness where the result is "[r]eached by means of routine procedures in the instant case. (Brackets and quotations in original.)

In connection with this Applicants note it appears the Examiner has conceded that a synergistic effect is enough to establish unobviousness. Therefore, the Examiner is requested to clarify on what grounds the previous response has been deemed to be non-persuasive in view of this statement. Furthermore, if the Examiner intends to rely on quoted excerpts in deeming the Applicants' arguments unpersuasive, the Examiner is courteously requested to indicate the source of the quotation. Nonetheless, Applicants agree that a synergistic effect may be enough to establish unobviousness, and note that the Federal Circuit has stated the following in respect of chemical inventions:

> When a chemical composition is claimed, a prima facie case of obviousness under § 103 may be established by the PTO's citation of a reference to a similar composition, the presumption being that similar compositions have similar properties.... One way for a patent applicant to rebut a prima facie case of obviousness is to make a showing of "unexpected results," i.e., to show that the claimed invention exhibits some superior property or advantage that a person of ordinary skill in the relevant art would have found surprising or unexpected. The basic principle behind this rule is straightforward -- that which would have been surprising to a person of ordinary skill in a particular art would not have been obvious. The principle applies most often to the less predictable fields, such as chemistry, where minor changes in a product or process may yield substantially different results.... *In re Soni*, 54 F.3d 746, at 750 (Fed. Cir. 1995).

In view of this, the *Soni* court stated:

> Mere improvement in properties does not always suffice to show unexpected results. In our view, however, when an applicant demonstrates substantially improved results, as Soni did here, and states that the results were unexpected, this should suffice to establish unexpected results in the absence of evidence to the contrary. *In re Soni*, at 751.

In the present case, Applicants have demonstrated substantially improved results, namely, that the invention improves reduction of neuronal damage from no appreciable effect when each component is used alone, to a significant and synergistic effect when the agents are combined. Further, the Applicants have stated that such results are unexpected (see the enclosed declarations from Michael Tager previously submitted to the present Examiner in connection with related applications). No evidence to the contrary is on the record, other than the Examiner's conclusory allegation that the cited references disclose chemicals with similar properties and therefore the invention is obvious. Thus, Applicants respectfully submit that the refusal to allow the present claims is squarely in opposition to the Federal Circuit's position in *In re Soni,* and the Examiner is therefore respectfully requested to remove the stated rejections and allow the claims.

Applicants submit the following additional arguments in support of the present application, which arguments are corroborated by the declarations of Michael Tager and are therefore not merely arguments of counsel.

Thiol-Disulfide Status versus GSH deficiency

Applicants respectfully submit that the Examiner appears to have equated the treating of a disturbance of thiol/disulfide status with the correction of glutathione (GSH) deficiency. However, these two conditions are distinct. Further, the references relied upon by the Examiner are primarily directed to free thiol content instead of total thiol content, as follows.

It is well recognized in the art that the thiol status in cells and tissue is defined as the sum of protein-bound and non-protein-bound thiol groups (i.e., see pages 1200 – 1202 in Schafer and Buettner, Free Radical Biol. Med. 2001, 30: 1191 – 1212). Thus, the total thiol content of a cell is the sum of free thiols and protein-bound thiols. Protein-bound thiols are mainly present in the form of proteins containing sulfhydryl groups (-SH groups). The protein-bound thiols are detected as intracellular proteins as well as membrane-bound proteins at the cell surface.

A typical example of a non-protein-bound species is gluthathione, which accounts for about 80 % of the free or non-protein-bound thiol-groups detectable in a cell. The predominant fraction of free thiol-groups is represented by protein-bound thiol-groups, an in particular the amino acid cysteine. These proteins are the main component in cellular development and differentiation processes, as well as in detoxification processes. It is recognized in the art that reduced gluthathione (GSH deficiency) occurs as the main intracellular free thiol compounds reaches an intracellular concentration between 2 – 10 mM, whereas the total concentration of protein-bound thiol groups is between 15 – 25 mM, as observed in erythrocytes (i.e., see Rossi et al., Biochim. Biophys. Acta, 1995, 1243:230-238, page 230, column 1). Thus, the content of free thiol groups adds to a total concentration of 12.5 mM, whereas protein-bound thiol groups are present in the cells up to a concentration of 25 mM. Thus, there is an excess of protein-bound thiol groups in a cell.

A thiol or a free thiol in a cell is typically discussed as an R-SH species, where R can be a protein, glutathione, cysteine, or another group. The thiol is present in its reduced form. If the thiol group undergoes oxidation (for instance, in the presence of an oxidizing agent), a disulfide according to the formula R-S-S-R is formed. However, disulfides are not capable of reducing damaging compounds that may be present in a cell. It is therefore desirable to keep the concentration of disulfides in a cell 100 to 1000 times

lower than the thiol-concentration. (The negative influence of a low thiol level on several disease patterns is discussed at pages 1-5 of the instant application.)

As noted above, it is well recognized that the physiological content of free thiols in cells and tissue is defined as the sum of protein-bound and non-protein-bound thiol groups (see pages 1200 – 1202 in Schafer and Buettner, Free Radical Biol. Med. 2001, 30: 1191-1212). On page 1200, column 2 the authors describe the concentration of protein-bound groups in cells and tissues as being much greater than that of reduced glutathione. They further state on page 1202, column 2, that the protein-bound SH groups can play a role in the antioxidant network of cells and thereby influence the redox-environment of the cell.

Further evidence of the reduced influence or contribution of free GSH on the total thiol status of a cell can be drawn from the fact that the thiol content of a cell is only reduced about 20 % after selective inhibition of the GSH synthesis (i.e., see Täger et al., FRBM, 29, 1160-1165, 2000). Measurements of the total thiol content in cell samples were conducted before and after the addition of buthionin sulfoximin (BSO) – a known inhibitor of the glutathione synthesis. Table 2, column 2, of the Täger et al. reference shows the thiol expression indices as calculated by the ratio of thiol content of BSO-treated (GSH depleted) and untreated samples. Depending on the measuring method that is used, it can be clearly seen that the inhibition of glutathione synthesis only reduced the total thiol expression to a rather small extent.

Experimentally, it is possible to determine the total amount of free thiol-groups in a cell or even the amount of a specific thiol-group-containing compound, e. g. glutathione. In the present invention, however, the total amount of free protein-bound and non-protein-bound thiol-groups was determined. In this regard, one embodiment of the present invention treats the disturbed thiol status of a cell or tissue caused by a disturbance of the total thiol content (i. e., a disturbance of the thiol-disulfide status) by co-administration of α-lipoic acid (and/or a salt thereof) and a glutathione metabolism effector such as ambroxole (and/or salt) or an ACE inhibitor such as captopril, enalapril, ramipril, etc.. Tables 1 and 2 and Figures 1a, b and 2a, b of the instant application show specifically the influence of a combination of α-lipoic acid and ambroxole, captopril, or

enalapril on the intracellular thiol status and the membrane-bound thiol status, respectively.

The references are directed to glutathione only

One problem the present invention addresses is that of a disturbance of cellular thiol/disulfide metabolism in neurodegenerative diseases. Typically, the disturbance of cellular thiol/disulfide metabolism results in extensive loss of normal cell functions, such as processes of cellular growth and differentiation including the programmed cell death as well as mechanisms of cell protection and cell decontamination. Medicaments according to the present invention can correct the disturbance of the thiol-disulfide metabolism and thus, are useful for the treatment of neurodegenerative diseases.

Jablonka et al. is silent with regard to the influence of ambroxol on the total thiol/disulfide status, not to mention any possible neuroprotective effect of ambroxol. Moreover, this reference does not disclose an effect of ambroxol on the total amount of free protein-bound and non-protein-bound thiol groups. In fact, on page 63 in the last paragraph of the discussion, the authors of the study state: "... on the basis of the methodology used it is impossible to say whether ambroxol stimulates the antioxidant enzyme production or presents the properties of the radical scavenger."

Stoll et al. relates to alpha-lipoic acid and provides general speculation that free radical damage may be involved in aging and age-related diseases like Alzheimer's disease and Parkinson's disease. However, the reference is silent with regard to the influence of alpha-lipoic acid on the total thiol/disulfide status and does not even utilize a relevant model of neurodegenerative disease; the reference employs normal animals to determine whether alpha-lipoic acid can improve the cognitive function of normal mice. Accordingly, there is no disclosure in this reference that alpha-lipoic acid can reduce or prevent neuronal damage.

Biewenga et al. refers to the antioxidant activity of alpha-lipoic acid and its reduced form DHLA; it merely provides a report of direct antioxidant activity by alpha-lipoic acid and its influence on GSH production metabolism. However, Biewenga et al. does not discuss the effect of alpha-lipoic acid on the total thiol/disulfide status, and in fact, Biewenga et al. makes no attempt to measure thiol/disulfide satus at all.

Kozhevnikova et al. demonstrates a neuroprotective effect in cerebral ischemia for the ACE inhibitors captopril and enalapril due to their hypertension-regulating properties. However, Kozhevnikova et al. does not discuss the influence of captopril and enalapril on the total thiol-disulfide status.

Sian et al. refers to alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders. However, as can be seen from page 350, right column et seq. under "Results" there is no correlation between GSH reduction and the tested neurodegenerative diseases. As further disclosed on page 351, right column "Glutathione Levels in Multiple-System Atrophy" the GSH content of the lateral globus pallidus from MSA patients was approximately double that found in control subjects. There were no significant changes in GSH or GSSG levels in other brain areas examined. In the group "Glutathione Levels in Huntington's Disease" the GSH content in cerebran cortex, caudate nucleus, and SN zona compacta from HD patients was not altered compared to control values. However, there was a marked reduction in the GSSG content. Thus, **Applicants respectfully submit therefore that the assumption of the Examiner that GSH level is low in neurodegenrative diseases is incorrect and is inconsistent with the references cited in support of this assumption**.

In view of the foregoing, Applicants submit it is clear that none of the cited references motivate, teach or even suggest the present invention, which is drawn to compositions and methods for achieving a synergistic protection from neurodegeneration. In particular, none of the references even contemplates the importance of the complete thiol level in a cell as characterized by the sum of all protein-bound and non-protein-bound thiol groups for a dysfunction of a cell or a tissue. Furthermore, none of the references provides any disclosure as to how to increase the total amount of free thiol groups in a cell or tissue.

There would be no reasonable expectation of success in achieving the present invention from the references

As discussed above, the synergistic effect of the present invention is demonstrated in a clinically relevant in vivo global ischemia gerbil model after OGD damage. The data in Figure 2 in Dr. Frank Striggow's previously submitted declaration clearly show the

influence of alpha-lipoic acid, ambroxol and enalapril and a combination of the three drugs on the neuronal damage after simulating a neurodegenerative event. As noted above, whereas the singular administration of alpha-lipoic acid, ambroxol and enalapril has **no** neuroprotective effect (the ranges of alpha-lipoic acid, ambroxol and enalapril are equal to the ranges of the OGD control group (neuronal damage after a standard OGD without the application of any substance neither alone nor in combination) the administration of the cocktail (three drugs in combination) caused a considerable reduction of the neuronal damage. Further, as can be clearly seen from Figure 2 of Dr. Frank Striggow's declaration, the neuronal damage is 15.9 ± 1 for the control OGD whereas the neuronal damage is 9.7 ± 0.6 for the combination Ambroxol + Enalapril and 9.0 ± 1.1 for the cocktail alpha-lipoic acid + Ambroxol + Enalapril. This means a reduction of the neuronal damage of 39 % for the combination Ambroxol + Enalapril ((15.9 – 9.7)/15.9 x 100 = 39 %) and a reduction of the neuronal damage of 43.4 % for the cocktail alpha-lipoic acid + Ambroxol + Enalapril ((15.9 – 9.0)/15.9 x 100 = 43.4 %). It is also evident from Figures 2, 3 and 4 the single components have almost no influence on the neuronal damage in said in vitro model. The same is also applicable from Figure 1 which shows the number of viable neuronal cells after OGD. In the case of "NaCl" which means the OGD event + the simultaneous application of NaCl solution, the number of living neuronal cells was only 17.5 ± 1.5. This value corresponds to 16.9 ± 3.5 for the OGD event + the application of Ambroxol. This means a reduction of the neuronal damage could not be achieved by the application of Ambroxol, but only for the combination Ambroxol + Enalapril or the cocktail alpha-lipoic acid + Ambroxol + Enalapril. Thus, these data, which provide technical support for the instant specification and particularly for Example 3, therefore demonstrate the synergistic effect according to the presently claimed method. As indicated in the declarations enclosed herewith, this is an unexpected finding.

Summary

As noted above, MPEP 2142 indicates that to establish a *prima facie* case of obviousness, three basic criteria must be met.

1) There must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings.

In the present case, based on the foregoing, Applicants submit that it is clear that no such motivation to combine the references can be found.

2) Second, there must be a reasonable expectation of success. Even assuming *arguendo* that the Examiner has identified a proper motivation to combine the references (an assumption which Applicants do not make), there record is utterly devoid of any evidence that one skilled in the art would have formed a reasonable expectation of success in achieving present invention, and in particular the synergistic aspects of the invention. Moreover, no evidence is on the record that one skilled in the art would have formed such an expectation *from the cited references.* However, and importantly, both the MPEP and the Federal Circuit require that the reasonable expectation of success must be found in the prior art, and not based on applicant's disclosure. *In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991)). In the instant case, no such evidence has been provided. Further, Applicants have demonstrated in the application as filed and through declaratory evidence that the invention functions to achieve a synergistic result, and have submitted declaratory evidence that the results would be unexpected to one skilled in the art. Thus, in view of *In re Soni*, which indicates that when an applicant demonstrates substantially improved results (as Applicant did here), and states that the results were unexpected (as Applicant did here), it is sufficient to establish unexpected results, in the absence of evidence to the contrary. Therefore, Applicants respectfully but vehemently reiterate that since no evidence that one skilled in the art would have formed a reasonable expectation of success in achieving the present invention from the cited references has been presented, Applicants respectfully submit that a *prima facie* case of obviousness has not been presented, and thus, the Examiner is compelled to remove the stated rejections.

3) The prior art references must teach or suggest all the claim limitations. None of the cited references disclose a composition comprising ambroxol or its salts and at least one inhibitor of the angiotension-converting enzyme. Moreover, none of the cited reference discloses administration of a composition comprising ambroxol or its salts and

at least one inhibitor of the angiotension-converting enzyme to achieve a synergistic inhibition of neuronal damage.

Thus, based on the foregoing, Applicants submit that a *prima facie* case of obviousness has not been presented for the present claims because one or more of the required elements for making such a case have not been presented.

Conclusion

Based on the arguments and amendments presented herein, Applicants believe all the pending claims are now in condition for allowance and respectfully request the Examiner to allow all the claims. Applicants request a two-month extension of time to file this response. A check for the required fee of $450.00 is enclosed. Please charge any additional fees due or credit any overpayment to deposit account number 08-2442.

Respectfully submitted,
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